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R 228 THE REDUCTION OF THE FREEZING POINT OF TOBACCO PLANTS TRANSFORMED WITH THE GENE ENCODING FOR THE ANTIFREEZE PROTEIN FROM WINTER FLOUNDER, JungSook Lee*[1], M. Selim Cetiner[2], William J. Blackmon[2], and Jesse M. Jaynes[1], Departments of Biochemistry[1] and Horticulture[2], Louisiana State University, Baton Rouge, LA 70803.

The winter flounder, *Pseudopleuronectes americanus*, can survive in seawater at temperatures below freezing by producing antifreeze proteins which depress the freezing point of their cellular fluids. The antifreeze gene encodes a 91 amino acid protein which is composed of a mature protein of 53 amino acids and a preproprotein of 38 amino acids. The gene encoding the mature antifreeze protein, including a start methionine, was placed under the control of the cauliflower mosaic virus 35S promoter residing on the binary vector pBI 121. In order to obtain enhanced expression, the gene was also introduced into a plasmid which would allow expression from the double 35S CaMV promoter and the construct subcloned into the intermediate vector pMON200 and the binary vector pBI 121. After triparental mating and infection of tobacco leaf-disks with *Agrobacterium tumefaciens* containing pBI 121-AF and pBI 121-fAF, transgenic plantlets were obtained which were kanamycin resistant and GUS positive. Southern analysis confirms the presence of single copy gene integration. Several individual plants were selected and tested for the reduction of the freezing point of leaf tissue by differential calorimetry. The data conclusively demonstrates the freezing point depression in transgenic plants of about 3-5°C compared to transformed controls. These results have confirmed the ability of this fish protein to confer increased frost-tolerance to plants.